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Removal of Mutagens from Drinking Water by Granular Activated Carbon: Evaluation Using Bacterial Mutagenicity Tests

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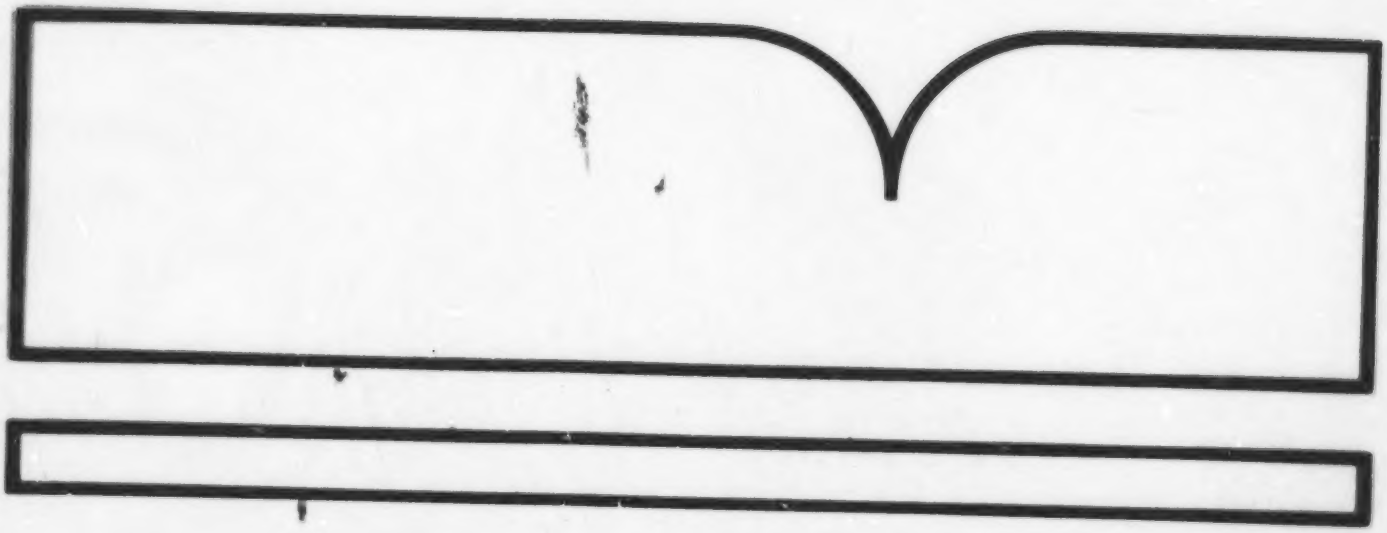

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16. ABSTRACT

The performance of a full-scale granular activated carbon (GAC) treatment system in removing mutagens from drinking water obtained from the Ohio River has been evaluated using two bacterial mutagenicity tests. The *Salmonella* microsome assay (Ames Test) and a fluctuation assay were both performed using *Salmonella typhimurium* strains TA98 and TA100. Influent and effluent waters were collected at two GAC adsorbers, one filled with virgin GAC and one with nearly exhausted GAC. The samples were submitted to reverse osmosis (RO) pre-concentration, sequential liquid liquid extractions and XAD-2 resin adsorption. The RO aqueous concentrations of both influents gave positive mutagenic responses with both strains in the fluctuation assay but no activity in the Ames test. The extracts and adsorbates showed mutagenic responses in the Ames test with both strains, the highest values being observed with TA100 in the absence of metabolic activation. The summation of mutagenic activity on the basis of net revertants per liter indicated that exhausted GAC removed a substantial fraction (more than 85%) of the mutagenic activity whereas virtually complete removal was observed with virgin GAC. These data suggest that short-term mutagenicity tests may be useful in evaluating the performance of GAC or other adsorbents used in the treatment of drinking water.

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REMOVAL OF MUTAGENS FROM DRINKING WATER BY GRANULAR ACTIVATED CARBON

EVALUATION USING BACTERIAL MUTAGENICITY TESTS

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Abstract—The performance of a full-scale granular activated carbon (GAC) treatment system in removing mutagens from drinking water obtained from the Ohio River has been evaluated using two bacterial mutagenicity tests. The *Salmonella*/microsome assay (Ames Test) and a fluctuation assay were both performed using *Salmonella typhimurium* strains TA98 and TA100. Influent and effluent waters were collected at two GAC adsorbers, one filled with virgin GAC and one with nearly exhausted GAC. The samples were submitted to reverse osmosis (RO) pre-concentration, sequential liquid–liquid extractions and XAD-2 resin adsorption. The RO aqueous concentrations of both influents gave positive mutagenic responses with both strains in the fluctuation assay but no activity in the Ames test. The extracts and adsorbates showed mutagenic responses in the Ames test with both strains, the highest values being observed with TA100 in the absence of metabolic activation. The summation of mutagenic activity on the basis of net revertants per liter indicated that exhausted GAC removed a substantial fraction (more than 85%) of the mutagenic activity whereas virtually complete removal was observed with virgin GAC. These data suggest that short-term mutagenicity tests may be useful in evaluating the performance of GAC or other adsorbents used in the treatment of drinking water.

INTRODUCTION

The discovery of mutagenic and carcinogenic organic compounds in numerous drinking water supplies has caused increasing concern with respect to hazards to human health (Loper, 1980; U.S. National Academy of Sciences, 1977, 1980b). In addition to chemicals originating from industrial contamination of source waters, disinfection practices, especially chlorination, have been found to be responsible for the introduction and/or production of mutagens and carcinogens in water (Bull *et al.*, 1981; Cheh *et al.*, 1980; de Greef *et al.*, 1980; Dolara *et al.*, 1981; Gruener, 1978; Loper, 1980; Maruoka & Yamanaka, 1980; Nestmann *et al.*, 1979a, b). The need to remove or control the presence of these potentially harmful compounds from drinking water supplies has intensified research into new and improved methods of water treatment.

Granular activated carbon (GAC) treatment is regarded as one of the best available methods for removing or effectively reducing the concentration of many water contaminants of health concern (Burke *et al.*, 1981; De Marco *et al.*, 1981; Dobbs & Cohen, 1980; Smith *et al.*, 1978; Symons, 1978; U.S. National Academy of Sciences, 1980a). To date, performance of GAC treatment has been studied primarily by chemical analyses (TOC, TOX, TTHM, trihalomethanes and specific organic compound analysis). However, the large majority of organic chemicals present in drinking water are not chemically identified. Compounds with mutagenic activity are known to exist among these non-characterized organics. (Loper *et al.*, 1978; Nestmann *et al.*, 1979a, b). To date there have been no studies which demonstrate the effectiveness of GAC treatment in removing these mutagens.

The *Salmonella*/microsome assay developed by Ames *et al.* (1975) is currently the most widely used and well validated short-term mutagenicity test (Ames, 1979; Bartsch *et al.*, 1980) and has been successfully applied to aquatic systems by several authors as an initial step in the evaluation of cancer hazards associated with drinking water consumption (Bull *et al.*, 1978; Dolara *et al.*, 1981; Forster & Wilson, 1981; Glatz *et al.*, 1978; Grabow *et al.*, 1980, 1981; Gruener, 1978; Gruener & Lockwood, 1979; Loper, 1978; Neeman *et al.*, 1980; Nestmann, 1979a). Besides this method, bacterial fluctuation assays have been used to test for the presence of mutagens in both unconcentrated and concentrated water samples (Forster & Wilson, 1981; Van Kreijl *et al.*, 1980). These assays have been reported to be more sensitive than the Ames test and have the added advantage that aqueous samples can be incorporated into the assay at much higher volumes (up to 80% of the incubation medium) than the Ames test (Green *et al.*, 1976, 1977a, b; Hubbard *et al.*, 1981; Kowbel & Nestmann, 1981; Levin *et al.*, 1981; Nestmann *et al.*, 1979b).

The present study has been undertaken to assess the performance of a full-scale GAC adsorption sys-

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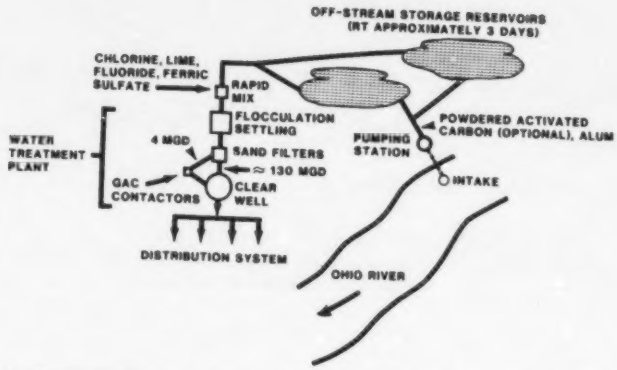

Fig. 1. Schematic diagram of the California Plant of the Cincinnati Waterworks.

tem in removing mutagens from drinking water using the *Salmonella*/microsome test and a bacterial fluctuation assay. The drinking water samplings were carried out at the California Plant of the Cincinnati Water Works (Cincinnati, Ohio) because this site is considered to be representative of a vulnerable water system that might require GAC treatment (De Marco *et al.*, 1981).

EXPERIMENTAL

Facilities and sampling descriptions

The California Plant of the Cincinnati Water Works normally processes 135 millions of gallons per day (mgd) of Ohio River water and consists of four sequential unit processes that include long-term presettling followed by coagulation-flocculation, sedimentation and rapid sand filtration. Aluminum sulfate is added to the raw water prior to presettling. Chlorine, lime, ferric sulfate and hydrofluorosalicic acid (for fluoridation) are applied between the presettling basin and sand filters. The treatment process is illustrated in Fig.1.

A system of four 1 mgd GAC adsorbers was used to treat the sand filtered water (post-filtration adsorption systems). The characteristics of the adsorbers are summarized in Table 1. Two of these contactors, one filled with virgin GAC and one with GAC which had been in use for about 3 months, were chosen for the samplings. The in-service contactor was nearly exhausted in its ability to retain organic material as indicated by measurements of Total Organic Carbon (TOC) (Table 2). Therefore, this contactor is referred to hereafter as the exhausted GAC contactor. A more complete description of the long term performance of these contactors is contained in an earlier publication (De Marco *et al.*, 1981). For each contactor, 7570 l. (2000 gallons) of the influent water (before GAC treatment) were sampled concurrently with 7570 l. of the effluent water (after GAC treatment). Each sample was collected in 50 gal. batches over a 1 week period. The individual batches were

Table 1. Characteristics of granular activated carbon (GAC) adsorbers

GAC data		Hydraulic data	
Manufacturer and type	Westvaco, WV-G	Diameter (ft)	11
Mesh size	12 × 40	Bed height (ft)	14.8
Total BET surface area ($m^2 g^{-1}$)	1100 minimum	Surface loading (gpm ft^{-2})	6.94
Mean particle diameter (mm)	0.90–1.20	Flow per day (mgd)	1
Carbon weight (lbs)	42,500	Residence time (min)	16

Table 2. Volumes and total organic carbon (TOC) values of drinking water samples concentrated by reverse osmosis

	Virgin GAC treatment		Exhausted GAC treatment	
	Influent	Effluent	Influent	Effluent
Initial water sample				
Volume (l.)	7570	7570	7570	7570
TOC ($mg l^{-1}$)	1.54	0.16	1.89	1.25
RO–cellulose concentrate				
Volume (l.)	30.3*	34.8*	32.5*	37.8*
TOC ($mg l^{-1}$)	167.9	5.71	211.1	83.9
RO–nylon concentrate				
Volume (l.)	37.5*	34.1*	34.4*	37.1*
TOC ($mg l^{-1}$)	18.8	1.91	34.1	33.7

*Approximately 10 l. of this volume was removed for mutagenicity and toxicity studies and was not processed by extractions and adsorption.

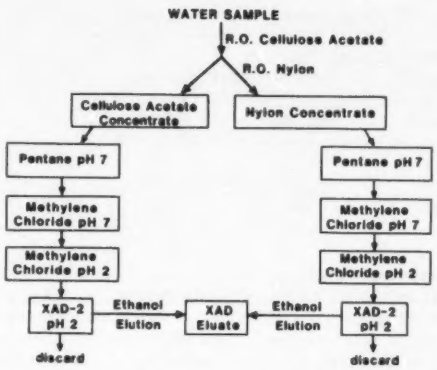

Fig. 2. Scheme of water concentrates and extracts preparation.

concentrated by reverse osmosis immediately following their collection and then pooled to yield a composite sample. The composite samplings of the influent and effluent of the exhausted GAC contactor were performed 2 weeks prior to the samplings of the influent and effluent of the virgin GAC contactor.

Preparation of concentrated organics from water

Each 75701. water sample was concentrated by a procedure utilizing reverse osmosis (RO), followed by liquid–liquid extractions and finally XAD-2 resin adsorption (Kopfler *et al.*, 1977a, b; Smith *et al.*, 1978). This concentration procedure is shown in Fig. 2.

The reverse osmosis system was used to produce about 200-fold concentrated aqueous solutions of organics, utilizing a combination of two different membranes. The water samples were first processed using a cellulose acetate membrane, then, the permeate from this step was reprocessed using a DuPont Permasep® nylon based membrane. Total organic carbon (TOC) measurements were carried out before, during, and after the RO concentration. From these values the percentage recoveries of organic carbon by the reverse osmosis process were calculated as follows:

$$\text{Recovery}\,(\%) = \frac{V_f C_f}{V_i C_i} \times 100$$

where:

C_i = initial TOC concentration
C_f = final TOC concentration
V_i = initial water volume
V_f = final water volume.

A portion of each aqueous concentrate (approx. 10 l.) was removed, filter sterilized using a 0.45 μm membrane and used for mutagenicity and carcinogenicity studies. The remaining portion was processed by three sequential liquid–liquid extractions and by XAD-2 resin adsorption, yielding seven different fractions for each water sample.

Liquid–liquid extractions of aqueous concentrates

1st extraction (pentane). The aqueous concentrates were first extracted with 70 ml of pentane l^{-1} with a mechanical stirrer. The pentane layer was separated from the aqueous layer, dried by adding anhydrous sodium sulfate, and the pentane extract was concentrated to about 2 ml by Kuderna–Danish evaporation.

2nd extraction (dichloromethane, pH 7). The aqueous phase was then extracted as before, using dichloromethane instead of pentane and the extracts were treated as before.

3rd extraction (dichloromethane, pH 2). Finally, the aqueous phase was adjusted to a pH 2 with concentrated HCl and then reextracted with dichloromethane as before.

XAD-2 resin adsorption of aqueous concentrates

The aqueous concentrates processed by the three sequential liquid–liquid extractions were stripped of pentane and dichloromethane with clean dry nitrogen, then each concentrate was passed over a 4.2 × 38 cm column containing 500 cm^3 of purified (Junk *et al.*, 1974) XAD-2 resin at a flow rate of about 70 cm^3 min^{-1}. The adsorbed organic material was eluted with 95% ethanol and the eluates of the two membrane concentrates were combined to yield the XAD-2 eluate fraction.

Salmonella/microsome assay

Tester strains. Because of the limited yields of some extracts and adsorbates obtained, the tests were performed only with the two plasmid bearing strains of *Salmonella typhimurium*, TA98 and TA100. These two strains have been found to be the most sensitive of the tester strains to compounds present in the aquatic environment (Glatz *et al.*, 1980; Grabow *et al.*, 1980, 1981; Loper, 1980). The strains were supplied to us by Dr B. Ames, Berkeley, California. Frozen permanents of broth cultures of the strains were stored at −80°C. In addition to frozen permanents, cultures on minimal agar plates containing histidine, biotin, and ampicillin were prepared and stored at 4°C. These master plates were used routinely as the source of inoculum for 16 h overnight cultures, grown in nutrient broth (Oxoid CM67) at 37°C in a shaking water bath.

Metabolizing system (S9). Male Sprague–Dawley rats of about 200 g weight were given a single i.p. injection of Aroclor 1254 in corn oil at a dose of 500 mg kg^{-1} and were sacrificed on the fifth day of induction. Liver post-mitochondrial supernatant (S9) and the S9 mix, containing 50 μl S9/ml^{-1}, were prepared by the procedure of Ames *et al.* (1975).

Preparation of test materials. The sterile RO aqueous concentrates were tested directly by adding up to 2 ml per plate, with and without metabolic activation (±S9). The extracts and adsorbates were concentrated to about 2 ml then portions were dried at room temperature in a desiccator and weighed at constant weight. Solutions in dimethylsulfoxide (DMSO) at different concentrations were prepared.

Controls. Negative controls consisted of distilled sterile water for aqueous concentrates and DMSO for the extracts. Tests for S9 and sample sterility were routinely carried out. In accordance with the recommendations of DeSerres & Shelby (1979) for the *Salmonella*/microsome mutagenesis assay, the following compounds were chosen as positive controls: sodium azide (1 μg) for TA100 without S9 activation; 2-nitrofluorene (1 μg) for TA98 without S9 activation; 2-aminoanthracene (1 μg) for both the strains with S9 activation. Toxicity of the samples to the bacteria was estimated by examination of the bacterial lawn under a microscope, and checking for the presence of characteristic pinpoint colonies or of the reduction of colony counts below the background.

Plate assay for mutagens. The standard plate method of Ames *et al.* (1975) was followed except that the agar concentration in the top agar solution was increased to 0.75% in order to test up to 2 ml volumes of the aqueous concentrates. Samples were tested in volumes of 100 μl per plate, in at least a 3-log range of concentration. Narrower ranges of concentrations were tested for obtaining dose–response curves.

Interpretation and expression of results. The criteria for positive results were the observations of a dose related response and a 2-fold increase in the number of induced revertants/plate over spontaneous revertants/plate values. "Specific activity" values were chosen for a rapid comparison of the mutagenic potential of each fraction and for computing the total mutagenic potential for each water sample. For this purpose, net revertants mg^{-1} values for each fraction were calculated by least-squares regression

Table 3. Percentage recovery of total organic carbon (TOC) by reverse osmosis concentration

Reverse osmosis concentrates	TOC recovery (%) Virgin GAC treatment		Exhausted GAC treatment	
	Influent	Effluent	Influent	Effluent
RO–cellulose	43.6	16.4	47.9	33.5
RO–nylon	6.0	5.4	8.2	13.2
Total	49.6	21.8	56.1	46.7

analysis of the linear portion of the dose–response curves. The total mutagenic potentials were computed as net revertants mg^{-1} of total fractions, by summing the net revertants mg^{-1} values contributed by each of the seven fractions after adjustment according to their percentage of the total weight (Epler *et al.*, 1978; *Guerin et al.*, 1981; Schoeny *et al.*, 1981). The total mutagenic potentials were also expressed as net revertants l^{-1} of original water, taking into account the total organic material recovered per liter of the original water and the calculated net revertants mg^{-1} of total fractions.

Fluctuation assay for mutagens

A fluctuation assay was performed using the *S. typhimurium* strains TA98 and TA100 according to a procedure being used at the Water Research Centre, Medmenham, U.K. (R. Forster, personal communication), which is a modification of the method described by Hubbard *et al.* (1981). 15 ml solutions were prepared with 2% Vogel Bonner medium (V–B medium), supplemented with glucose (0.4%), biotin (10 μl ml^{-1}), histidine (1.5 $\mu g\ ml^{-1}$ for TA98 and 0.5 $\mu g\ ml^{-1}$ for TA100), and with 30 μl of overnight culture (approx. 3×10^7 cells for TA98 and 3×10^6 cells for TA100). The aqueous concentrates were incorporated into these solutions by making up the V–B medium with filter sterilized sample and a concentrate of VB salts. 300 μl aliquots of these bulk solutions were dispensed into forty-eight 2 ml capacity wells of a 96 well plastic tray which had been sterilized by gamma-irradiation (Linbro, Flow Laboratories Ltd). The trays were incubated for 16–18 h at 37°C after which 1 ml per well of a selection medium (2% Vogel Bonner medium supplemented with 0.4% glucose and 5.0 $\mu g\ ml^{-1}$ bromcresol purple) was added. The trays were incubated for an additional 72 h and then scored by counting the number of wells showing bacterial growth, as indicated by a change to acid pH (yellow color). A typical test consisted of a negative control (bidistilled sterile water), a positive control, and a series of concentrations of the sample. The statistical evaluation of the individual results was performed using a one-tailed Chi-square test, holding the error rate for the testing of the results involving the same negative control to 0.05 (i.e. experimentwise error rate = 0.05). A trend analysis was also done on the data for which there appeared to be a dose response relationship between the number of positive wells and the amount of sample (Maxwell, 1971). The overall Chi-square test for a given treatment dose combination was partitioned into a test for a linear relationship between dose and the number of positive wells, and a test for lack of fit of this regression line. This subdivision of the overall Chi-square test is a more powerful and sensitive analysis than the individual Chi-square test.

RESULTS

Recovery of organic material

Table 2 lists the volumes of water processed in producing the aqueous concentrates and subsequent extracts, and the TOC concentrations at various stages of the reverse osmosis process. The percentage recoveries of organic carbon by this process are listed in Table 3.

Table 4 lists the concentrations which were calculated from residue weights, and the percentage composition of the fractions (extracts and adsorbates)

Table 4. Calculated concentrations per liter of original water and percentage of fractions obtained from reverse osmosis concentrates by liquid–liquid extractions and resin adsorption

Fractions	Concentration* ($\mu g\ l^{-1}$) and percentage							
	Virgin GAC treatment				Exhausted GAC treatment			
	Influent		Effluent		Influent		Effluent	
	($\mu g\ l^{-1}$)	(%)	($\mu g\ l^{-1}$)	(%)	($\mu g\ l^{-1}$)	(%)	($\mu g\ l^{-1}$)	(%)
RO–cellulose extracts								
Pentane	0.5	0.12	0.4	8.58	1.9	0.19	0.5	0.11
Dichloromethane (pH 7)	10.8	2.50	0.2	4.73	24.2	2.38	2.5	0.56
Dichloromethane (pH 2)	41.8	9.67	1.0	21.60	78.4	7.73	6.8	1.50
Total RO–cellulose extracts	53.1	12.29	1.6	34.91	104.5	10.30	9.8	2.17
RO–nylon extracts								
Pentane	1.3	0.31	0.2	4.14	0.9	0.09	0.3	0.06
Dichloromethane (pH 7)	4.0	0.93	0.1	2.66	3.4	0.33	3.3	0.74
Dichloromethane (pH 2)	2.3	0.53	0.4	8.00	25.9	2.55	4.3	0.96
Total RO–nylon extracts	7.6	1.77	0.7	14.80	30.2	2.97	7.9	1.76
XAD-2 adsorbates	371.2	85.94	2.2	50.29	879.8	86.72	433.3	96.06
Total fractions	431.9	100.00	4.5	100.00	1014.5	100.00	451.1	100.00

*Values corrected for the volumes of water removed for mutagenicity and toxicity studies.

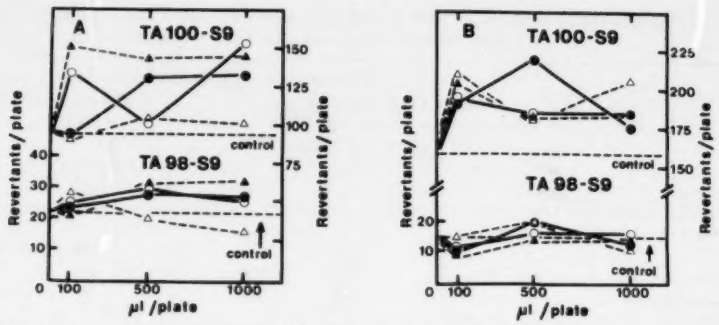

Fig. 3. Mutagenic activity of two sequential reverse osmosis (RO) aqueous concentrates from drinking water samples treated by GAC. (A) RO–cellulose aqueous concentrates. (B) RO–nylon aqueous concentrates. Virgin GAC treatment: influent (▲) and effluent (△). Exhausted GAC treatment: influent (●) and effluent (○).

obtained by liquid–liquid extractions and by resin adsorption from aqueous concentrates. The adsorbates of all the water samples constituted the largest portions (50.3–96.0%) among all the fractions, followed by the total RO–cellulose extracts (2.2–34.9%) and finally by the total RO–nylon extracts (1.8–14.8%). Among the organic solvent extracts the dichloromethane extractions at pH 2 of both aqueous concentrates usually yielded the largest quantities. It is notable that overall recovery of organic material differed substantially between samples. The recoveries were calculated using the data in Tables 3 and 4 and were expressed as the dry weight recovered per the TOC content of the original water samples. Recoveries were found to be 0.28 and 0.54 for the influents to the virgin and exhausted GAC, respectively; and 0.03 and 0.36 for the effluents from the virgin and exhausted GAC, respectively. The poor recovery for the virgin GAC effluent indicates some common chemical features are shared by those chemicals removed by GAC and those effectively concentrated by reverse osmosis and/or XAD-2.

Salmonella/microsome assay

RO aqueous concentrates. The RO aqueous concentrates of each water sample were tested up to a dose of 2 ml/plate, but volumes larger than 1 ml showed formation of spreading colonies, difficult to count. Mutagenic responses less than 2-fold the background were obtained for all the samples with both strains either in the presence or absence of metabolic activation (±S9). Even though increases in mutation rate were observed for the two aqueous concentrates with TA100 without S9, no clear dose–responses were evident (Fig. 3). These findings are most likely due to insensitivity of the assay to the low volumes of original water corresponding to each ml of aqueous concentrate (about 200 ml) and to the low concentrations of organic material present in them (1.9–2.11.1 g l^{-1} TOC).

Extracts of RO–cellulose concentrates. The influents and effluents obtained from the GAC contactors differed markedly in their mutagenic activity (Tables 5 and 6). The pentane extract was uniformly negative. However, mutagenic activity was consistently found in the neutral and acidic dichloromethane extracts of the influent waters with both strains TA98 and TA100. Figures 4, 5 and 6 illustrate the dose–response curves for mutagenic activity obtained with these samples.

Mutagenic activity (net revertants mg^{-1} of isolated material) in both dichloromethane extracts (pH 7 and 2) was reduced to nondetectable levels with both TA98 (Table 5) and TA100 (Table 6) after virgin GAC treatment. In contrast, the specific mutagenic activity detected in the influent to the GAC contactor which had been on line for about 3 months was substantially reduced only in the neutral dichloromethane extract (completely in the case of TA100 and about 60% for TA98). The specific activity observed in TA100 with the acidic dichloromethane extract was reduced by only 30% and remained essentially the same with TA98 after exhausted GAC treatment.

Extracts of RO–nylon concentrates. Removal of mutagenic activity recovered in the RO–nylon extracts followed the same general pattern as that observed with the RO–cellulose extracts. In this case, however, amounts of material recovered in certain fractions following GAC treatment were too small to allow thorough testing. Where testing could be conducted, treatment using virgin carbon consistently resulted in decreases in specific mutagenic activity. A detectable level of TA98 positive activity did remain in the neutral dichloromethane extract (Table 5). As with material recovered by the cellulose acetate membrane significant levels of mutagenic activity were detected in TA98 with both neutral and acidic dichloromethane extracts following treatment with exhausted carbon. Overall the specific activity in the exhausted carbon effluent was still reduced substantially (about 60%) relative to the influent.

XAD-2 adsorbates. As previously noted these adsorbates constituted the highest amounts of organics among the total fractions. Positive results were observed only with TA100 without S9 in both influent water samples (Fig. 7, Tables 5 and 6).

Table 5. Specific mutagenic activity with strain TA98 in the *Salmonella*/microsome assay of the fractions derived from drinking water samples before and after GAC treatment

Fractions	Net revertants mg l^{-1} with strain TA98*							
	Virgin GAC treatment				Exhausted GAC treatment			
	Influent		Effluent		Influent		Effluent	
	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
RO–cellulose extracts								
Pentane	NS	NS	NS	NS	NS	NS	NS	NS
Dichloromethane (pH 7)	48	32	NS	NT	59	38	24	19
Dichloromethane (pH 2)	96	43	NS	NS	80	40	76	33
RO–nylon extracts								
Pentane	NS	NS	NS	NT	NS	NS	NS	NT
Dichloromethane (pH 7)	36	45	25	NT	51	34	21	18
Dichloromethane (pH 2)	62	35	NS	NS	55	36	19	NS
XAD-2 adsorbates	NS	NS	NS	NS	NS	NS	NS	NS

*The values were calculated by a least-square regression analysis of the linear portion of the dose–response curve and are mean values of duplicate tests. The mean values of spontaneous revertants/plate (±SD) for all the tests carried out in this research were: −S9 = 17 (±3.8); +S9 = 23 (±3.7). The mean values of revertants/plate for positive controls (±SD) were: 2-nitrofluorene (1 μg) −S9 = 537 ± 149; 2-aminoanthracene (1 μg) +S9 = 1001 ± 219.

NS—not significant as indicated by no dose response and/or less than 2-fold increase over the spontaneous revertants rate.

NT—not tested, because of the low amount of fraction.

Total mutagenic potential. Total mutagenic potentials expressed as net revertants mg^{-1} of total fractions and net revertant l^{-1} of original water are shown in Tables 7 and 8. The net revertants l^{-1} data were computed from the net revertants mg^{-1} of total fractions values, taking into account the μg l^{-1} of total fractions present in the water samples (Table 4).

Fluctuation assay

Since the results with the Ames test were negative for the reverse osmosis aqueous concentrates but positive, in some cases, for fractions derived from these samples, it seemed important to verify that the mutagenic activity was not due to artifacts produced by the fractionation procedure. Therefore, the reverse osmosis samples were retested for mutagenic activity using a fluctuation assay. This method was selected since it has been successfully used to detect mutagens at low concentrations (Green *et al.*, 1976; Hubbard *et al.*, 1981). The results with this assay are presented in Table 9. Statistical analyses of the data using a one-tailed Chi-square test revealed that significant mutagenic activity was detected with strain TA100 in both RO–cellulose GAC influent samples and in the RO–

Table 6. Specific mutagenic activity with strain TA100 in the *Salmonella*/microsome assay of the fractions derived from drinking water samples before and after GAC treatment

Fractions	Net revertants mg^{-1} with strain TA100*							
	Virgin GAC treatment				Exhausted GAC treatment			
	Influent		Effluent		Influent		Effluent	
	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
RO–cellulose extracts								
Pentane	NS	NS	NS	NS	NS	NS	NS	NS
Dichloromethane (pH 7)	91	95	NS	NT	115	106	NS	NS
Dichloromethane (pH 2)	934	160	NS	NS	479	112	332	104
RO–nylon extracts								
Pentane	NS	NS	NT	NT	NS	NS	NS	NT
Dichloromethane (pH 7)	129	97	NT	NT	103	89	NS	NS
Dichloromethane (pH 2)	198	135	NS	NS	164	115	NS	NS
XAD-2 adsorbates	131	NS	NS	NS	94	NS	NS	NS

*Values were calculated by a least-square regression analysis of the linear portion of the dose–response curve and are mean values of duplicate tests. The mean values of spontaneous revertants/plate (+SD) for all the tests carried out in this research were: −S9 = 123 + 28.9; +S9 = 129 ± 22.0.

The mean values of revertants/plate for positive controls (±SD) were: sodium azide −S9 (1 μg) = 1327 ± 232; 2-aminoanthracene +S9 (1 μg) = 1275 ± 418.

NS—not significant as indicated by no dose response and/or less than 2-fold increase over the spontaneous revertants rate.

NT—not tested, because of the low amount of fraction.

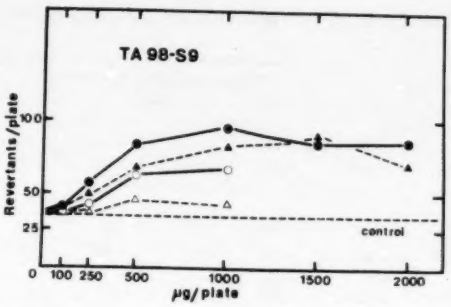

Fig. 4. Mutagenic activity with strain TA98 of dichloromethane extracts (pH 7) of RO–cellulose aqueous concentrates from drinking water treated by GAC. Virgin GAC treatment: influent (▲) and effluent (△). Exhausted GAC treatment: influent (●) and effluent (○).

nylon exhausted GAC influent sample. No significant activity was detectable in any of the effluent samples. Although no significant responses were observed for any of the samples with strain TA98, there did appear to be a dose-related increase in the number of positive wells for the RO–cellulose influent samples using this strain. When the data for the RO–cellulose influent samples were further analyzed for statistical significance by a trend analyses (Table 10), in every case, except for the results with TA98 on the virgin GAC influent, Chi-squared values due to regression were significant. This finding indicates that there was a significant increase in the number of positive wells with increasing sample amount. The fact that there was non-significant depature from regression indicates a linear dose–response relationship for the samples. Recently Forster *et al.* (1981) have reported that problems with artifactual results may be encountered with

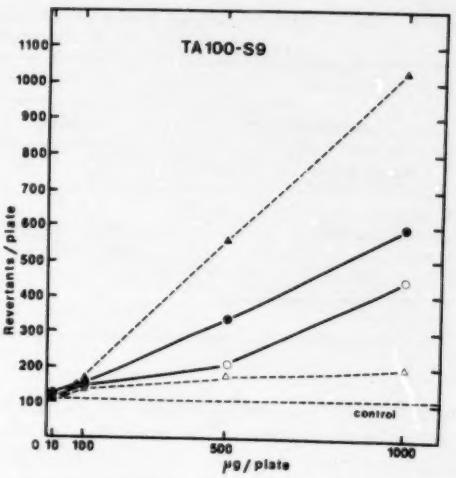

Fig. 6. Mutagenic activity with strain TA100 of RO–cellulose dichloromethane extracts (pH 2). Virgin GAC treatment: influent (▲) and effluent (△). Exhausted GAC treatment: influent (●) and effluent (○).

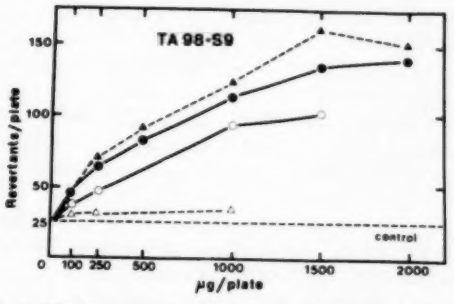

Fig. 5. Mutagenic activity with strain TA98 of dichloromethane extracts (pH 2) of RO–cellulose aqueous concentrate. Virgin GAC treatment: influent (▲) and effluent (△). Exhausted GAC treatment: influent (●) and effluent (○).

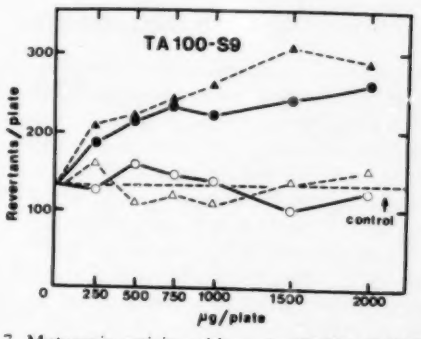

Fig. 7. Mutagenic activity with strain TA100 of XAD-2 adsorbates. Virgin GAC treatment: influent (▲) and effluent (△). Exhausted GAC treatment: influent (●) and effluent (○).

Table 7. Total mutagenic potentials per mg of total fractions and per liter of original water observed with strain TA98 in the *Salmonella* microsome assay

Total mutagenic responses with strain TA98	Virgin GAC treatment				Exhausted GAC treatment			
	Influent		Effluent		Influent		Effluent	
	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Net revertants mg^{-1} of total fractions	11.1	5.6	0.7	NS	9.2	4.7	2.6	0.7
Net revertants l^{-1} of water	4.8	2.4	<0.1	<0.1	9.3	4.8	1.2	0.3
Minimum volume (l.) of water showing positive response	3.5	9.6	>240	>230	1.8	4.8	14.2	76.7

Table 8. Total mutagenic potentials per mg of total fractions and per liter of original water observed with strain TA100 in the *Salmonella*/microsome assay

Total mutagenic responses with strain TA100	Virgin GAC treatment				Exhausted GAC treatment			
	Influent		Effluent		Influent		Effluent	
	−S9	+S9	−S9	+S9	−S9	+S9	−S9	+S9
Net revertants mg^{-1} of total fractions	207.4	19.5	NS	NS	125.8	14.4	5.0	1.6
Net revertants l^{-1} of water	89.2	8.4	<0.5	<0.5	127.6	14.6	2.3	0.7
Minimum volume (l.) of water showing positive response	1.4	15.4	>250	>250	1.0	8.8	53.5	184.3

the fluctuation assay when testing unconcentrated water or sewage samples. The problems, especially with false positive responses, apparently are due to the presence of histidine or other nutritional factors in the samples which may stimulate the growth of the bacteria. Typically when this situation is encountered no dose–response relationship is seen. However, in the present study dose related increases in responses were observed for all the positive results, except for the RO–nylon influent sample tested with TA100. Furthermore, the bacteria from the positive wells always showed growth when plated onto minimal agar without histidine (data not shown) confirming the occurrence of his− to his+ mutations.

DISCUSSIONS AND CONCLUSIONS

The present investigation demonstrates the utility of the bacterial mutagenesis tests under study in assessing the impact of water treatment processes on mutagenic substances in drinking water. Others have demonstrated the widespread occurrence of direct-acting mutagenic chemicals in drinking waters (Nestmann *et al.*, 1979a, b; Dolara *et al.*, 1981; Glatz *et al.*, 1978; Grabow *et al.*, 1981; Loper *et al.*, 1978). In this study we have shown that a full-scale GAC treatment system efficiently removes direct-acting mutagens from drinking water.

The results of TOC analyses (Table 2) showed that about 90% of the organic material present in the influent water was removed by the virgin GAC. In contrast, the efficiency of total organics removal was only 34% with the GAC which had been in service for 3 months. These findings are similar to those reported by De Marco *et al.* (1981).

The method used in this study for concentrating and isolating the organic material from drinking water has been recommended by Jolley (1981) for concentrating large quantities of non-volatile organics. The procedure was chosen because of its utility in processing large quantities of water and because it would enable us to isolate mutagens into different fractions, thus allowing qualitative as well as quantitative comparisons of the four water samples. Large water volumes were needed in order to obtain sufficient quantities of organic material for valid test comparisons. This was especially critical in the case of the virgin GAC treated water which contained only one-tenth the level of organics that were present in the influent water (Table 2). The recovery of non-volatile organics with this method has been found by Kopfler to be 35–40% (Kopfler *et al.*, 1977a, b). This figure compares very favorably with XAD-2 alone which has been reported to give 5–20% recovery (Forster *et al.*, 1981). On the other hand, low molecular weight volatile compounds such as the trihalomethanes are generally poorly recovered by the reverse osmosis method (F. Kopfler, personal communication).

Differences in recovery of organics were evident among the four water samples at the various stages of concentration and isolation. For example, following the 200-fold volume reduction by the reverse osmosis process, approx. 50% of the total organic carbon was recovered for all the samples except for the virgin GAC treated water, where only a 22% recovery was obtained (Table 3). These differences undoubtedly reflect the fact that recovery efficiencies are dependent to some extent on the types of compounds being concentrated (Fang & Chian, 1976) and their relative proportion among the total organics present. Studies examining specific compound removal by GAC have shown that some compounds are adsorbed by GAC with high efficiency while others have little or no affinity for it (De Marco *et al.*, 1981; Suffet *et al.*, 1978). As a result the proportion of certain compounds may be drastically altered by the GAC treatment and this will be reflected by changes in recovery efficiencies.

The preliminary concentration of organics by reverse osmosis enabled us to study directly the mutagenicity of concentrated aqueous samples. No mutagenic activity was detected using the standard Ames plate assay in any of these reverse osmosis aqueous concentrates. This finding was not unexpected since a calculation of the total mutagenic potential (Table 8) of even the most active of the samples (i.e. exhausted GAC influent) showed that 1 l. of the original water, or 5 ml of RO–concentrate would have been required to give a positive assay response. However, mutagenic activity was detected using a modified bacterial fluc-

Table 9. Induced mutation in strains TA98 and TA100 in the fluctuation assay by reverse osmosis aqueous concentrates

Sample	Volume of sample (ml)	TA98-S9				TA100-S9			
		RO–cellulose		RO–nylon		RO–cellulose		RO–nylon	
		Pos. wells (per 48)	Signif.*	Pos. wells (per 48)	Signif.*	Pos. wells (per 48)	Signif.*	Pos. wells (per 48)	Signif.*
Negative control	0	11		14		24		24	
Positive control†		45		43		47		48	
Virgin GAC treatment									
Influent	0.3	10	—	13	—	27	NS	22	—
	1.5	17	NS	19	NS	21	—	21	—
	3.0	22	NS	11	—	33	NS	18	—
	6.0	14	—	15	NS	39	$P < 0.001$	21	—
Effluent	0.3	11	—	14	—	23	—	28	NS
	1.5	12	NS	14	—	16	—	25	NS
	3.0	15	NS	11	—	20	—	23	—
	6.0	5	—	9	—	0	—	23	—
Exhausted GAC treatment									
Influent	0.3	10	—	13	—	21	—	31	NS
	1.5	14	NS	8	—	23	—	36	$P < 0.01$
	3.0	15	NS	15	NS	38	NS	25	NS
	6.0	18	NS	8	—	43	$P < 0.001$	26	NS
Effluent	0.3	10	—	14	—	27	NS	29	NS
	1.5	11	—	11	—	27	NS	24	—
	3.0	9	—	9	—	29	NS	23	—
	6.0	15	NS	9	—	27	NS	18	—

*Significance was calculated using one-tailed Chi-square analysis, holding the experimentwise error rate to 0.05.
†2-Nitrofluorene (0.1 $\mu g\ ml^{-1}$) for TA98-S9 and sodium azide (0.1 $\mu g\ ml^{-1}$) for TA100-S9.
NS—not significant; —not tested using Chi-square since values were lower or equal to control.

Table 10. Trend analysis* of the results of the fluctuation assays performed on RO–cellulose acetate aqueous concentrates of the two influents

Sample	TA98-S9			TA100-S9		
	d.f.†	Chi-square	Signif.	d.f.	Chi-square	Signif.
Virgin GAC treatment—influent						
Due to regression	1	1.42	NS	1	13.69	$P < 0.001$
Depature from regression	3	7.84	$P < 0.05$	3	4.43	NS
Overall	4	9.26	$P < 0.06$	4	18.12	$P < 0.002$
Exhausted GAC treatment—influent						
Due to regression	1	3.87	$P < 0.05$	1	24.22	$P < 0.001$
Depature from regression	3	0.36	NS	3	2.69	NS
Overall	4	4.23	NS	4	26.91	$P < 0.001$

*See Maxwell (1971).
†Degrees of freedom.
NS—not significant.

tuation assay in both influent RO–cellulose samples (Tables 9 and 10). This finding indicates an advantage in the mutagen detection capability of the fluctuation assay over the Ames test, which is a geniune advantage for this application. More importantly, the fact that mutagens were detected in the aqueous concentrates provides evidence that the mutagens detected in the fractions were not produced during the fractionation process. Additional evidence that mutagenic artifacts were not introduced during the concentration and isolation steps is apparent from the absence of mutagens in the virgin GAC effluent fractions.

The extraction and adsorption procedures following the RO concentration step permitted the characterization and comparison of the mutagenicity of seven different fractions for each water sample (Tables 5 and 6). The fractions obtained from the influents (before GAC treatment) showed mutagenic activity with both strains. The highest levels were observed with strain TA100 in the absence of metabolic activation (−S9), demonstrating that direct-acting mutagens are primarily involved. Fractions from the effluents showed complete removal (virgin GAC) or a substantial decrease (exhausted GAC) of the mutagenicity observed in the influent extracts. The XAD-2 adsorbates, which contained the greatest proportion of organics among the fractions, produced mutagenic responses only for the influents and only in TA100 without metabolic activation.

One question which we hoped to resolve by this study was whether the compounds which failed to adsorb to the GAC contactors were the ones possessing the highest mutagenic activity. For this to be true, the effluent fractions should have possessed higher specific mutagenic activity (net revertants mg^{-1}) than the corresponding influent fractions since the removal of organics by the GAC would be essentially a mutagen purification step. As is evident from tables 5 and 6, this was not found to be the case for either exhausted GAC or virgin GAC. Conversely, in most cases the specific mutagenic activities were actually lower in the effluents. This finding indicates a selective removal of mutagenic compounds by the GAC. It is notable that this selective reduction appears to be maintained by exhausted GAC.

The summation of the total mutagenic activities of the fractions according to their percentage weight made it possible to compare the overall activities of the four drinking water samples. This comparison (Tables 7 and 8) revealed that the full-scale GAC systems described here removed mutagens very efficiently. Even after three months in service, the removal of mutagenic activity was still greater than 87% despite the fact that the efficiency of TOC removal had been reduced to 34% (Table 2). Although the exhausted GAC contactor was somewhat less effective than the virgin GAC contractor for removal of mutagens, this may be partly due to the presence of higher levels of mutagens in the exhausted GAC influent (Fig. 8), which was sampled 2 weeks prior to the virgin GAC. The issue of how much longer the GAC can be used and still maintain this efficiency must await further studies with multiple samplings over a longer time span. Such biological test information should prove helpful in making cost vs benefit decisions on the frequency of replacement or regeneration of GAC. Studies employing chemical analytical techniques have identified a number of important factors which influence the adsorption characteristics of GAC. These include the type of GAC, regeneration of GAC, the GAC bed-life, desorption effects from chemical spills, and the quality of the influent water (Lee *et al.*, 1981; De Marco *et al.*, 1978; Suffet *et al.*, 1980; Yohe, *et al.*, 1981). Undoubtedly these variables as well as others will have to be considered in an overall assessment of the usefulness of GAC treatment.

Finally, a brief discussion of the meaning of the bacterial mutagenicity test data in relation to human health risks seems appropriate. Positive results in bacterial test systems, such as the Ames test and the fluctuation test, are generally regarded as indicative of potential genetic and carcinogenic hazards to human health. This interpretation is based primarily on the finding that the ability of chemical to cause mutation to DNA in bacteria is highly correlated (85–90%) with

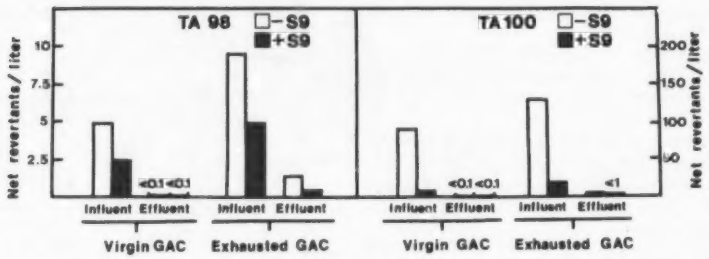

Fig. 8. Total mutagenic activity with strains TA98 and TA100 of drinking water samples, before and after GAC treatment.

the chemical's ability to cause cancer in animals (McCann & Ames, 1977; Sugimura *et al.*, 1976). While being good qualitative indicators of carcinogenic risk, these *in vitro* test systems do not take into account the absorption, distribution, and excretion of chemicals which occur *in vivo*, and they only approximate the *in vivo* metabolism of compounds. Because of these and other limitations inherent in *in vitro* test systems, we do not recommend that the mutagenicity data obtained here be used to make definitive conclusions regarding quantitative risks to human health and, in particular, the ability of GAC to reduce those risks. Rather the results from this study should be regarded as qualitative evidence for the reduction of potential genetic and carcinogenic hazards through the use of GAC treatment.

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